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Immunocompromise syndrome in homosexual men

Prevalence of possible risk factors and screening for the prodrome using an accurate white cell count

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SUMMARY The immunocompromise syndrome in homosexual men in the USA is thought to be associated with cytomegalovirus (CMV) infection and nitrite intake. Such men often have a lymphopenia. In a clinic in London 76% of 46 unselected homosexual men and 50% of 76 heterosexual controls had serum CMV IgG antibody at a titre of 1/16 or more ($p < 0.01$). No case of excretion of CMV in the urine was found. Thirty per cent of the homosexual men admitted to using nitrites.

These figures suggest that this population of homosexual men uses nitrites less often than their counterparts in the USA and is less likely to have evidence of active or past CMV infection. In addition, these male homosexual patients seem to be less promiscuous than those reported from the USA. Mean accurate total and differential white blood cell counts, using the Haemalog D automatic white cell counter, were no different in homosexual men (and various at risk subgroups of them) than heterosexuals, suggesting that the prodrome to the immunocompromise syndrome was not present in the London clinic population.

Introduction

The immunocompromise syndrome (ICS) in homosexual men has received much attention lately.[1 2] It is characterised by T lymphocyte dysfunction, commonly with lymphopenia which may lead to serious often fatal opportunistic infections and Kaposi's sarcoma (KS). The cause of the syndrome is not obvious, but present or previous cytomegalovirus (CMV) infections are prime factors.[3] CMV in homosexual men is, however, not a new or seemingly serious condition, so there has been a search for other causative factors. So-called recreational drugs, such as amyl nitrite and isobutyl nitrite, have been implicated. Current data suggest that nitrites may be immunosuppressive when associated with repeated viral antigenic stimulation and may contribute to the high frequency of the ICS and KS in homosexual men.[4] Whatever the causes of this syndrome, many of the patients complain of non-specific ill health before the onset of the serious illness.[5 6] The purpose of this study was to assess the prevalence of these risk factors in apparently healthy homosexual men and to look for lymphopenia in those patients at risk using the Haemalog D automated differential counter, which is an extremely accurate method of counting peripheral white blood cells.

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Patients and methods

All consecutive new male patients seen at the department of genitourinary medicine, University College Hospital, London, were entered into the survey. Those who were not exclusively homosexual or heterosexual were excluded. Consent of the hospital's ethics committee had previously been obtained. Details of each patient's age, number of sexual partners in the last four weeks, sexual preference, length of time that present level of sexual activity had been taking place, and the use of recreational nitrite drugs were recorded.

All patients had a general examination followed by an examination of the anogenital area. The presence of urethral discharge, penile or anal ulcers, and anogenital warts were noted. Urethral or rectal material or both was examined microscopically and

cultured for *Neisseria gonorrhoeae*. Serum from ulcers was examined by darkfield microscopy for *Treponema pallidum* and cultured for *Herpesvirus hominis*. A freshly passed stool specimen from all homosexual men was routinely examined for enteroparasites. Blood specimens were tested for hepatitis A and B and for syphilis by the *T pallidum* haemagglutination assay and rapid plasma reagin test and used to estimate the peripheral white blood count and antibody to CMV. A midstream specimen of urine was obtained from each patient for culture for CMV.

CMV URINE CULTURE

Freshly voided untreated urine was inoculated directly on to human embryo lung fibroblast cells and incubated for two hours at 36°C. The urine was then replaced by fresh Eagles BME medium with 2% fetal calf serum and the cells reincubated at 36°C. All specimens were examined daily for six weeks for the characteristic cytopathic effect of CMV.

CMV SEROLOGY

Sera were examined using the indirect immunofluorescence technique of Weller and Coons[7] and Hanshaw.[8] Human foreskin cells infected with CMV and fixed on glass slides (supplied by Electro-Nucleonics Inc) were treated with a 1/16 dilution of the patient's serum for 30 minutes at 37°C. This was washed off and replaced with FIIC conjugated antihuman IgG antiserum counterstained with rhodamine. After further incubation the cells were washed again, dried, and mounted before examination under an ultraviolet microscope.

Because of the induction of receptors to the Fc fragment of IgG molecules serum should be considered reactive to CMV only if there is fluorescence in the inclusion body formed in the nucleus of CMV-infected cells.[9] In this study, only those sera that reacted thus were considered to contain antibody to CMV.

HAEMATOLOGICAL INVESTIGATION

Five millilitres of blood were transferred to a Sequestrene container (Labco Ltd). Total and differential white cell counts were obtained using the Haemalog D counter.

This counter has the advantage over manual differential counting as 10 000 cells instead of the usual 100 cells are analysed. This eliminates the enormous sampling error inherent in manual techniques. For example, if 100 cells are counted and 50% of the cells are truly lymphocytes, then the percentage counted will vary between 40% and 60% (mean±250). With smaller percentages the range becomes considerably wider and assessment of minor cell populations such as eosinophils and basophils is extremely inaccurate.[10] The Haemalog D automated differential counter counts cells in each of three separate cytochemical staining channels. Details of the technique have been published elsewhere.[11 12] In the peroxidase channel each cell is assessed for its size and peroxidase activity as it passes through an optical detector and is automatically assigned to a given cell category. Lymphocytes mainly appear as small leucocytes with weak staining, though a few lymphocytes are larger cells and are recorded as large unstained cells. Neutrophils are of larger size with moderate peroxidase activity and eosinophils have very strong peroxidase activity. Monocytes and basophils are counted in separate staining channels using non-specific esterase for the enumeration of monocytes and Alcian blue for basophils.

RECORDING OF DATA

We postulated that a large number of recent sexual partners, nitrite use, and the presence of serum CMV antibodies were all risk factors for the development of the ICS and might decrease the peripheral lymphocyte count. We therefore calculated separately the mean total and differential white cell counts for: (A) homosexual men with a history of nitrite use (14) and (B) homosexual men with serum CMV IgG antibody and more than two recent sexual partners (14) and compared them with the mean white cell counts of control groups (heterosexuals and homosexuals without the above risk factors, whom we expected to have normal lymphocyte counts). Ideally, the mean count of patients in group B would have compared best with the group of men who were theoretically at lowest risk of developing the ICS (that is, with CMV-IgG-negative serum and less than two recent partners). Since there were only two patients in this group a comparison was made with the homosexual men who were serum CMV-IgG-negative, irrespective of the number of recent partners (9).

STATISTICAL ANALYSIS

Comparison of means of values for demographic data and differential white cell counts were performed by Student's t test. Numbers in groups positive for CMV IgG antibody were compared by the χ^2 test using Yates's correction.

Results

Seventy-seven heterosexual men and 46 homosexual men were seen in the survey. Details of age, current diagnosis of sexually transmitted disease, and sexual history are given in table I. Fourteen (30%) of 46 homosexual men admitted to using nitrites in the previous five years.

TABLE I *Details of age, sexual activity in recent past, and current STD diagnosis in 77 heterosexual and 46 homosexual men*

	Clinical group	
	Heterosexuals	*Homosexuals*
Age at presentation (years):		
Mean	30·9 (NS)	33·0 (NS)
SD	9·17	8·0
Range	19-66	19-52
No of sexual partners in last four weeks:		
Mean	1·54*	3·45*
Range	0-12	0-20
Median	2	3
No of years practising heterosexual or homosexual intercourse:		
Mean	12·6 (NS)	13·8 (NS)
Range	0·5-30	0·5-38
SD	9·2	9·2
Current diagnosis:		
Non-gonococcal urethritis	43	10
Gonorrhoea	5	9
Genital warts	1	5 (2 anal)
Herpes genitalis	1	1
Syphilis	2	0
Hepatitis A	0	1
Hepatitis B	0	0
Amoebiasis	ND	1
No disease	37	25
Total	89†	52‡

ND = Not done; SD = Standard deviation; NS = No significant difference
*$t = 5·0$; $p<0·001$
†In 77 patients
‡In 48 patients

No patient had unexplained fever, weight loss, generalised lymphadenopathy, skin or mucosal lesions suggesting KS, or appeared clinically anaemic. CMV was not isolated from the urine of any patient. CMV IgG antibody was present in 35 (76%) of 46 homosexual men. Serum specimens from two homosexual men showed incomplete inclusion immunofluorescence, but for purposes of comparison they were assumed to be positive. Of 76 heterosexual men antibody was found in 38 (50%). The difference between the two groups was statistically significant ($\chi^2 = 7·06$; $p<0·01$).

The mean total white cell, lymphocyte, and monocyte counts in the various subgroups of patients are given in table II. There was no statistically significant difference between the mean counts in any of the patient subgroups, except for the mean monocyte values in the serum CMV-IgG-negative homosexuals ($0·27 \times 10^9$/l) compared with the mean monocyte count of the serum CMV-IgG-positive homosexuals who had had more than two sexual partners in the preceding four weeks ($0·45 \times 10^9$/l) ($t = 2·45$; $p<0·05$). Mean counts for neutrophils, basophils, eosinophils, and large unstained cells in all the patient subgroups were similar.

Discussion

Cases of the immunocompromise syndrome are still being steadily reported to the Centers for Disease Control in the USA.[13] They have not yet been described in the UK, though a case of KS in a homosexual man with normal immunological indices has been recently described.[14] Social and virological factors are thought to be associated with the ICS. Inhalation of nitrites was admitted by over 80% of homosexual men attending sexually transmitted diseases clinics in New York, San Francisco, and Atlanta.[15] The rate of nitrite inhalation among homosexual men attending such clinics in London varies from over 80% at St Mary's hospital[16] to 30% in this department. Drew *et al*[17] found that 7% of homosexual men in a sexually transmitted diseases clinic in San Francisco excreted CMV in their urine; in contrast we did not find viruria in any of our 46 homosexual men. Serum antibody against CMV was found by Drew *et al*[17] in 94% of homosexuals and 54% of heterosexuals compared with our rates of 76% and 50% respectively.

TABLE II *Comparison of means of white blood cell counts in heterosexual and various groups of homosexual men*

Patient group (No in group)	*Total white cell count (× 109/l)*	*Lymphocytes (× 109/l)*	*Monocytes (× 109/l)*
Normal range	4-11	1-2·7	0·15-0·78
Heterosexuals (57)	5·85	1·70	0·33
Homosexuals (42)	6·34	1·68	0·43
Homosexual nitrite users (14)	6·65	1·73	0·37
Serum CMV-IgG-negative (irrespective of No of partners (9)†	5·51	1·51	0·27*
Serum CMV-IgG-positive and more than two partners in last four weeks (14)	6·35	1·77	0·45*

*Differences in means of total white cell count and differential counts in the various clinical subgroups not significant except for monocytes where $t = 2·43$; $p<0·05$
†Only two patients were IgG-negative and had less than two partners in the previous four weeks

In the present study there was essentially no difference in the mean total or differential white cell counts between the homosexual and heterosexual men, between the nitrite users and non-users, or between homosexual men with CMV IgG antibody who were promiscuous (a group which we considered to be, at least in theory, at high risk of developing the ICS) and any other subgroup of patients that we studied. Based on these accurate white cell counts there is no evidence to suggest that the prodromal stage of the ICS is present in homosexual men attending this clinic or that past CMV infection, as reflected by the presence of CMV IgG antibody, exposure to nitrites, or promiscuity are factors which have an appreciable effect on white cell count in the homosexual men in our study.

It is often assumed that homosexual men attending STD clinics are a homogeneous population the world over. There is evidence, however, that the sexual practices and diseases seen in patients attending clinics differ from one to another. *Chlamydia trachomatis* seems to be more often associated with proctitis in homosexual men in Seattle than in London. Lymphogranuloma venereum has not recently been described as a cause of proctitis in homosexual men in the UK, whereas in the USA this has been frequently reported.[18] The prevalence of serum markers for hepatitis B in homosexual men is known to vary according to where the patient presents, so that the rate has been much higher in the USA "gay bars" than in "evening meeting groups".[19] The present survey shows that our patients used nitrites less often than American homosexuals. There is even variation in this rate between clinics in London, so that patients at St Mary's Hospital used nitrites more often than our patients. The zero isolation rate of CMV from urine and the lower prevalence of serum CMV antibodies in our homosexuals compared with Drew's series from San Francisco[16] again highlights the heterogeneity of the group. Possibly, the British homosexual is less promiscuous and less likely to use nitrites than his American counterpart. This speculation may go some way to explain why an epidemic of the ICS has not yet occurred in the UK.

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References

1. Anonymous. Opportunistic infections and Kaposi's sarcoma in homosexual men. *N Engl J Med* 1981;**305**:1465-7.
2. Anonymous. Immunocompromised homosexuals (Editorial). *Lancet* 1981;ii:1325-6.
3. Gottleib MS, Schorff R, Schanker HM, *et al. Pneumocystis carinii* pneumonia and mucosal candidiasis in previously homosexual men: evidence of a new acquired cellular immunodeficiency. *N Engl J Med* 1981;**305**:1425-31.
4. Goedert JJ, Neuland CY, Wallen WC. Amyl nitrite may alter T lymphocytes in homosexual men. *Lancet* 1982;i:412-5.
5. Masur H, Michels MA, Greene JB, *et al.* An outbreak of community acquired *Pneumocystis carinii* pneumonia: initial manifestation of cellular immune dysfunction. *N Engl J Med* 1981;**305**:1431-8.
6. Siegal FP, Lopez C, Hammer GS, *et al.* Severe acquired immunodeficiency in male homosexuals, manifested by chronic perianal ulcerative herpes simplex lesions. *N Engl J Med* 1981;**305**:1439-44.
7. Weller TH, Coons AH. Fluorescent antibody studies with agents of varicella and herpes zoster propagated in vitro. *Proc Soc Exp Biol Med* 1954;**86**:789-94.
8. Hanshaw JB. Congenital cytomegalovirus infections. Laboratory methods of detection. *J Pediatr* 1969;**75**:1179-85.
9. Keller R, Peitchel R, Goldman JN, *et al.* An IgG Fc receptor induced in cytomegalovirus infected human fibroblasts. *J Immunol* 1976;**116**:772-7.
10. Dacie JV, Lewis SH. *Practical haematology,* 4th ed. London: Churchill Livingstone, 1968.
12. Ansley H, Orstein L. Enzyme histochemistry and differential white cell counts in the Technicon Haemalog D. In: *Advances in automated analysis.* Fifth Technicon International Congress. New York: Futura Publishing Company 1970; 437-6.
12. Patterson KG, Cawley JD, Goldberg AH, *et al.* A comparison of automated cytochemical analysis and conventional methods in the classification of acute leukaemia. *Clin Lab Haematol* 1980;**2**:281-91.
13. Anonymous. Persistent generalised lymphadenopathy among homosexual men. *Morbidity and mortality weekly reports.* Atlanta, Georgia: Centers for Disease Control. 1982;**31**: 249-51.
14. Maurice PDL, Smith NP, Pinching AJ. Kaposi's sarcoma with a benign course in a homosexual. *Lancet* 1982;i:571.
15. Anonymous. Special report: epidemiological aspects of the current outbreak of Kaposi's sarcoma and opportunistic infections. *N Engl J Med* 1982;**386**:248-52.
16. McManus TJ, Starrett TA, Harris JRW. Amyl nitrite use by homosexuals. *Lancet* 1982;i:503.
17. Drew WL, Mintz L, Miner RC. Prevalence of cytomegalovirus infection in homosexual men. *J Infect Dis* 1981;**143**:188-192.